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MINIREVIEW

A Biochemical Mechanism for Nonrandom Mutations and Evolution

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As this minireview is concerned with the importance of the environment in directing evolution, it is appropriate to remember that Lamarck was the first to clearly articulate a consistent theory of gradual evolution from the simplest of species to the most complex, culminating in the origin of mankind (71). He published his remarkable and courageous theory in 1809, the year of Darwin's birth. Unfortunately, Lamarck's major contributions have been overshadowed by his views on the inheritance of acquired characters. In fact, Darwin shared some of these same views, and even Weismann (106), the father of neo-Darwinism, decided late in his career that directed variation must be invoked to understand some phenomena, as random variation and selection alone are not a sufficient explanation (71). This minireview will describe mechanisms of mutation that are not random and can accelerate the process of evolution in specific directions. The existence of such mechanisms has been predicted by mathematicians (6) who argue that, if every mutation were really random and had to be tested against the environment for selection or rejection, there would not have been enough time to evolve the extremely complex biochemical networks and regulatory mechanisms found in organisms today. Dobzhansky (21) expressed similar views by stating "The most serious objection to the modern theory of evolution is that since mutations occur by 'chance' and are undirected, it is difficult to see how mutation and selection can add up to the formation of such beautifully balanced organs as, for example, the human eye."

The most primitive kinds of cells, called progenotes by Woese (108), were undoubtedly very simple biochemically with only a few central anabolic and catabolic pathways. Wächterhäuser (105) theorizes that the earliest metabolic pathway was a reductive citric acid cycle by which carbon fixation occurred (64). At that point in time, some four billion years ago, how did the additional, more complex metabolic pathways found in even the simplest prokaryotes evolve? For that matter, how are they evolving today? As pointed out by Oparin (79), it is inconceivable that a self-reproducing unit as complicated as a nucleoprotein could suddenly arise by chance; a period of evolution through the natural selection of organic substances of ever-increasing degrees of complexity must intervene. Horowitz (40) suggests a plausible scheme by which biosynthetic pathways can evolve from the successive depletion and interconversion of related metabolites in a primitive environment, as the rich supply of organic molecules is consumed by a burgeoning population of heterotrophs. Thus, a possible scenario begins with the starvation of a self-replicating unit for its precursor, metabolite A, utilized by enzyme 1 encoded by gene 1. When metabolite A is depleted, a mutation in a copy of gene 1 gives rise to gene 2 and allows enzyme 2 to use metabolite B by converting it to metabolite A. Then metabolite B is depleted, obtained from metabolite C, and so on, as an increasingly complex biochemical pathway evolves. In fact, there are examples in which a similar series of events can actually be observed in the laboratory, for example, involving enzymes that are "borrowed" from existing pathways, via regulatory mutations, to establish new pathways (75).

The starvation conditions that may initiate a series of events such as those described above target the most relevant genes for increased rates of transcription, which in turn increase rates of mutation (111). Transcriptional activation can result from the addition of a substrate or from the removal of a repressor or an end product inhibitor. The latter mechanism, called derepression, occurs in response to starvation for an essential substrate or for an end product that represses its own synthesis by feedback inhibition. Since evolution usually occurs in response to stress (41), transcriptional activation via derepression is the main focus of this minireview.

EVOLUTION OF BIOCHEMICAL PATHWAYS

A number of events initiated by carbon source starvation can facilitate the evolution of a new catabolic pathway. Under these circumstances, cells with gene duplication and higher enzyme levels have a selective advantage (87, 95). In some systems, duplicated segments are specifically subject to higher mutation rates (93), providing ideal and expendable material for mutations representing minor modifications of existing genes (58). These new genes can encode modified enzymes catalyzing reactions closely related and/or complementary to those in existence (56). An additional consequence of starvation is the removal of feedback controls, resulting in the derepression of genes previously inhibited by the now absent metabolite. Increased rates of mutation in these derepressed genes increase the probability of creating a new gene-enzyme system. A number of examples exist in which derepression of a gene has enabled an enzyme to use a new substrate. For example, altros-galactosidase can be used by β-galactosidase after it is derepressed (53); other examples are β-glycerophosphate via alkaline phosphatase (100), putrescine via diamine-α-ketoglutarate transaminase (44), and d-mannitol via d-arabitol dehydrogenase (55).

An excellent example of the evolution of biochemical pathways involves the modification of two genes to serve the new demands imposed by carbon source starvation (56, 112). Ribitol dehydrogenase, which is induced by ribitol in wild-type
Enterobacter aerogenes (strain X in Table 1), is unable to use xylitol. Starvation for ribitol in the presence of xylitol results in a mutation to strain X1, in which ribitol dehydrogenase is constitutive and able to use xylitol, which is a poor substrate for the enzyme but not its inducer. By repeated growth cycling on xylitol, derivative mutants X2 and X3 are obtained with lower K_m for xylitol. The enhanced uptake of labeled xylitol in the final mutant, X3, is due to the acquisition of a constitutively expressed active transport system for xylitol, originating from the modification of an inducible transport system for D-arabitol. Thus, two preexistent gene-enzyme systems evolve to initiate a new catabolic pathway in response to the stress of imminent starvation.

In the evolution of a growth rate-limiting amino acid biosynthetic pathway, starvation, derepression, and higher mutation rates can result in a lower K_m for the rate-controlling endogenous precursor of the pathway or in the ability to use a new and more plentiful precursor for the synthesis of that amino acid.

**SPECIFICITY OF STARVATION-INDUCED DEREPRESSION**

Starvation for any essential nutrient activates systems that protect the vulnerable cells from environmental damage (37, 72, 91). In addition, elaborate and specific feedback mechanisms are deployed that counteract the particular crisis created by the absent nutrient (9, 13, 39, 54, 66, 77). For example, inorganic phosphate (P_i) starvation derepresses the pho regulon, including a new high-affinity P_i transport system able to cope with lower phosphate levels, and a hydrolase able to obtain P_i from new sources (67). Nitrogen starvation derepresses the ntr regulon, including glutamine synthetase, which has a higher affinity for NH_4^+ than the constitutive glutamate dehydrogenase (65). Starvation for leucine specifically targets the leu operon (111). Regulation of amino acid biosynthetic operons by attenuation is exquisitely sensitive (over ranges of 1,000-fold) to the need for, and the supply of, all the amino acids (18, 49, 97). Attenuation regulation is an impressive example of the remarkable mechanisms that have evolved to ensure the conservation of precious reserves and the derepression and activation of only those systems essential for survival under particular conditions of starvation. As seen in Table 2, each amino acid operon encodes in its leader sequence a series of codons for the amino acid product of that operon. This sets the stage for a very complex and specific mechanism to monitor the precise amount of amino acid required relative to the amount available (49, 107). If the Leu codons are replaced with Thr codons, regulation of the leu operon by leucine is abolished (12).

Presumably, feedback mechanisms existing today evolved in the past to prevent unnecessary and wasteful metabolic activities by coordinating these activities with the presence or absence of nutrients in the environment. High mutation rates in derepressed genes prepare cells to respond rapidly to new challenges should the stress become more severe. As will become apparent, genetic derepression may be the only mechanism by which particular environmental conditions of stress target specific regions of the genome for higher mutation rates (hypermutation). Although this direct avenue for increasing variability is probably not available to multicellular organisms in which germ cells and somatic cells are separated, the derepression of biosynthetic pathways is essential to increased longevity in mammals subjected to caloric restriction (54), and amino acid limitation in rats can also induce gene expression (9).

**MECHANISMS OF MUTATIONS VERSUS MECHANISMS OF EVOLUTION**

In a scientific context, the word spontaneous is meaningless. Every event is preceded by, and dependent upon, innumerable known and unknown prior events and circumstances. Therefore, the work background will be used when referring to the many environmental conditions, cellular events, and repair processes that affect mutation rates in nature. Although background mutations, such as deamination, alkylation, and depurination, occur with low frequencies, they have characteristic, finite activation energies under physiological conditions (31, 32, 48, 60), and the molecular mechanisms by which they occur are well established. For example, protonation of the N-3 of cytosine results in its deamination to uracil. This process occurs 140 times more frequently in single-stranded DNA (ssDNA) than in double-stranded DNA (dsDNA) (in which N-3 is hydrogen bonded to N-1 of guanine), in mismatched base pairs, and in AT-rich regions that “breathe.” These background mutations and frameshift-induced deletions and additions are DNA sequence directed in that they occur most frequently in ssDNA and in unpaired, mispaired, and methylated bases (15, 26, 28, 31, 32, 60). Such vulnerable bases can arise as a consequence of slippage in tandem repeats or as a result of stem-loop DNA secondary structures that arise from sequences containing intrastrand inverted complements. The loops in these structures consist of unpaired bases particularly vulnerable to mutations (other more complex structures containing vulnerable bases are also important as precursors of mutations but will not be discussed in this minireview).

In an evolutionary context, we are not concerned with the above molecular mechanisms by which individual types of mutation occur but with another kind of mechanism. Evolution depends upon events that enhance mutation rates, thus increasing the supply of variants from which the fittest are selected. Therefore, the word mechanism in the present context will refer to the circumstances affecting mutation rates. That any DNA-destabilizing event will increase mutation rates is evidenced by the increasing frequency of mutations in genes and operons that are subject to these influences (106). The variety of these effects is many and complex, and the resulting types of mutations exhibit an infinite diversity. In this account, the emphasis will be placed on those mutations that arise through events having a specific, deterministic character. The methods of analysis and the results of actual experiments show that the various determinants of mutation rate do not act independently in the genome but together create a complex system of self-regulating interactions (13).

**TABLE 1. Evolution of a catabolic pathway**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Generation time (h)</th>
<th>K_m (mM)</th>
<th>_[^14C]_xylitol uptake (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X⁶</td>
<td>4.1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>X1⁷</td>
<td>4.1</td>
<td>290</td>
<td>3</td>
</tr>
<tr>
<td>X2</td>
<td>1.7</td>
<td>120</td>
<td>2</td>
</tr>
<tr>
<td>X3</td>
<td>0.9</td>
<td>130</td>
<td>2</td>
</tr>
</tbody>
</table>

⁶ Data taken from reference 112.
⁷ Wild-type strain with inducible ribitol dehydrogenase.

**TABLE 2. Leader sequences of attenuation-regulated operons**

<table>
<thead>
<tr>
<th>Operon</th>
<th>Amino acid sequence of codons in leader RNA⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>his</td>
<td>Met-Thr-Arg-Val-Gln-Phe-Lys-His-His-His-His-</td>
</tr>
<tr>
<td>phe</td>
<td>His-Pro-Asp-Met-Lys-His-Ile-Pro-Phe-Phe-Phe-</td>
</tr>
<tr>
<td>thr</td>
<td>Met-Lys-Arg-Ile-Ser-Thr-Thr-Ile-Thr-Thr-Thr-</td>
</tr>
<tr>
<td>leu</td>
<td>Met-Ser-Ile-Thr-Ile-Val-Thr-Thr-Thr-Thr-Thr-</td>
</tr>
</tbody>
</table>

⁴ Operon-specific sequences shown in bold type.
axiomatic. In growing cells, such events include polymerase and proofreading errors during DNA replication, as well as recombination, transcription, and repair. DNA transcription and repressor binding affect the rate of deletions in *Escherichia coli* plasmids (101). The availability of ssDNA (leading- and lagging-strand DNA templates) facilitates the slippage of tandem repeats and the formation of stem-loop structures (89). In nongrowing cells, however, the DNA-destabilizing events of replication are probably not primary causes of mutations. Within an hour following starvation, bacterial cells undergo major metabolic transitions (the stringent response [13]) in which genes required for cell division are repressed while a number of other genes (depending upon the starvation regimen) are derepressed. During this transition from exponential growth to stationary phase, events related to gene activation parallel a sharp increase in supercoiling, suggesting that transcriptional activation may drive supercoiling and the resulting DNA secondary structures that are precursors of mutations (discussed below). Among the known DNA-destabilizing events, only transcription can be selectively activated (7), either by induction or derepression. Derepression of the *leu* operon in *E. coli* is specifically correlated with an increased rate of *leu*B mRNA turnover (62; J. M. Reimers, A. Longacre, and B. E. Wright, Conf. DNA Repair Mutat., abstr. B29, p. 80, 1999) and an increased reversion rate of the *leu*B mutant gene; this mutation is located at the site of a predicted stem-loop structure (111).

**RANDOM VERSUS NONRANDOM HYPERMUTATION**

As discussed above, background mutations are sequence directed and not random in the sense that they occur in bases made vulnerable by virtue of their particular location within specific DNA sequences, such as tandem repeats, or the unpaired and mispaired bases of stem-loop structures. Dobzhansky's statement (22) enlarges upon this point: "The structure of a gene is a distillate of its history, and the mutations that may occur in a gene are determined by the succession of environments in which that gene and its ancestors existed since the beginnings of life. The environment prevailing at the time mutation takes place is only a component of the environmental complex that determines the mutation." The definitions of directed and random that are appropriate in the above context are neither relevant nor useful, however, when discussing mechanisms of evolution. By the neo-Darwinian definition, a mutation is random if it is unrelated to the metabolic function of the gene and if it occurs at a rate that is undirected by specific selective conditions of the environment. For example, mutagenic DNA-destabilizing events associated with cell division are random, as they are dependent upon growth rate and selective conditions of the environment only insofar as those conditions affect the rate of cell division. However, the focus of this minireview concerns the consequences of environmental stress on evolution. What are the DNA-destabilizing processes operative in stressed, nongrowing organisms forced to mutate before they can continue to multiply? Mechanisms must have evolved in starving cells to stimulate metabolic changes and mutations that facilitate adaptation to new circumstances.

With the above neo-Darwinian definition of random in mind, an impressive array of circumstances that enhance background mutation rates in response to environmental stress may be examined with respect to whether or not they are random (undirected). Examples of conditions that result in undirected, genomewide hypermutation include those caused by UV irradiation, reactive oxygen species, mismatch repair-deficient mutants, horizontal gene transfer by transduction with a viral particle, and mobile genetic elements that increase mutation rates by inserting at particular regions or at target sequences within the genome (73, 76). Such mechanisms are undirected because, for example, a mismatch repair deficiency will result in failure to repair a particular kind of lesion regardless of whether or not it confers a selective advantage upon its host.

In higher organisms, environmental conditions of stress do not have direct access to the cells involved in reproduction, and different mechanisms resulting in hypermutation have evolved. For example, localized DNA rearrangements and shuffling produce extensive beneficial variation (82, 96), and hypervariable sequences provide continual changes in the composition of venoms produced by snakes (29) or snails (78) to overcome resistance developed by their predators or prey. These mechanisms are also random. The threat of predators does not result in hypermutation; there is no evidence that the circumstances selecting such hypermutable genes bear any metabolic relationship to the mechanisms by which they originally arose. A gene may be hypermutable because it contains a hot spot due to a particular DNA sequence, and if a high mutation rate is advantageous to its host, that gene will be selected during evolution. However, its hypermutability per se is undirected, since it is unrelated to those selective conditions and to the function of the gene. These random mechanisms resulting in hypermutation are in essence serendipitous relationships; in contrast, hypermutation resulting from derepression is localized as a direct consequence of a specific response to environmental challenge.

**TWO MECHANISMS BY WHICH TRANSCRIPTION CAN INCREASE MUTATION RATES**

**Transcription exposes ssDNA.** The most common base substitution events in the spectra of background mutations in *E. coli* and mammalian cells are G·C-to-A·T transitions. Fix and Glickman (28) observe that 77% of these mutations originate on the nontranscribed strand in *E. coli* mutants unable to repair deaminated cytosines. This suggests that the unprotected single strand in the transcription “bubble” is significantly more vulnerable to mutations than the transcribed strand, which is protected as a DNA-RNA hybrid (Fig. 1A). The frequency of UV-induced lesions in the *lacI* gene is also higher in the nontranscribed strand than in the transcribed strand (46). In fact, cytosines deaminate to uracils in ssDNA at more than 100 times the rate in dsDNA (31, 32, 60). The relative mutability of the nontranscribed strand is also seen in a plasmid system in which a fourfold increase in the frequency of transitions occurs selectively in the nontranscribed strand when transcription is induced (4). Transcription may therefore be a prerequisite for many C-to-T transition mutations, since other mechanisms resulting in the transient generation of single-stranded sequences, such as replication or breathing (102) do not lead to asymmetry in the two strands. Apparently, the observed strand bias cannot be explained by transcription-coupled repair (36), since base mismatches are poor substrates for this kind of repair, and the same strand bias is observed when the host is deficient in repairing U·G and T·G mismatches (4). Thus, transcription may be implicated as a major cause of background transition mutations in nature.

Transcriptional activation as a mechanism for increasing mutation rates was first proposed in 1971, by Brock (8) and Herman and Dworkin (38). Their work demonstrates that *recA*-independent *lac* reversion rates of frameshift and point mutations are higher when transcription is induced by isopro-
pyl-β-δ-thiogalactopyranoside (IPTG), and that the effect is specific. More recently, specifically induced, transcription-enhanced mutations have also been shown for a lys frameshift mutation in Saccharomyces cerevisiae (16, 74). Starvation-induced stringent response mutations in E. coli (62, 109–111) and Bacillus subtilis (90) occur as a result of transcriptional activation triggered by gene derepression, not induction. In this system, mutations arise during the transition between growth and stationary phase and they are recA independent, similar to the lac reversions mentioned above. This distinguishes them from prolonged stress-induced adaptive mutations (11) and from DNA damage-induced SOS mutagenesis (104), both of which require recA (and will not be discussed in this minireview). It is noteworthy that the experiments described above on the effects of artificially induced transcription on mutation rates in growing cells are all examples of specifically directed mutations. However, none of the researchers come to that conclusion or challenge the assumptions and implications inherent in the experiments of Luria and Delbruck (63), which reinforce neo-Darwinism. This situation may be due to the dominance of current dogma and to the assumption that mechanisms operative during growth cannot also be critical during evolution under conditions of environmental stress. In fact, the limited evidence now available suggests that only growing cells, or cells in transition between growth and stationary phase, have the metabolic potential required for specific, transcription-induced mutations in response to environmental challenge. Thus, IPTG induction enhances lac reversion rates in growing cells (38) but not in cells subjected to prolonged stress (17). Transcriptional activation is the mechanism for enhancing mutation rates both in the artificially induced systems and in stringent response mutations (90, 111; J. M. Reimers, A. Longacre, and B. E. Wright, Conf. DNA Repair Mutag., 1999). However, only the latter are relevant to evolution, since they occur naturally as a result of starvation-induced derepression. Mutations that most benefit organisms and accelerate evolution may occur as an immediate response to imminent starvation, when cells still have the metabolic resources to respond specifically to the particular conditions of stress at hand.

**Transcription drives localized supercoiling.** Chromosomal DNA from bacterial cells is negatively supercoiled. The level of global negative supercoiling in E. coli cells is maintained within a physiologically acceptable range by two opposing enzyme activities: DNA gyrase, which introduces negative supercoils, and topoisomerase I, which relaxes them. Investigations with plasmids grown in E. coli (59, 81) demonstrate the presence of stem-loop structures in naturally occurring supercoiled circular DNA molecules (Fig. 1B). Analyses with single-strand-specific nuclease show that DNA molecules with high superhelical densities are selectively cleaved, in contrast to their linearized counterparts with which they are in dynamic equilibrium in vivo. The sequence surrounding the area of cleavage reveals inverted complementary sequences that hydrogen bond to become the stem separated by noncomplementary bases that become the single-stranded loop and the substrate for nuclease cleavage. Such complex structures form preferentially in easily denatured AT-rich stretches of DNA and occur about 10,000 times more frequently than expected by chance (30, 59), suggesting their selection during evolution. Data indicate that stem-loop-based recombination may have evolved in the early “RNA world” (94) and that the potential to generate stem-loops was later conserved, for example, in hypervariable snake venom genes under strong selection to keep one step ahead of both predators and prey (29).

A number of variables, such as temperature, anaerobiosis, osmolarity, and nutritional shifts, affect DNA supercoiling (1, 19, 47, 85, 86). Some environmental perturbations affect plasmid systems and chromosomes in a similar manner, while some apparently do not (25). Transcription both responds to and promotes changes in supercoiling. The optimal level of supercoiling for gene expression varies for different genes, and su-

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**FIG. 1.** (A) Exposure of the nontranscribed strand during transcription; (B) effect of transcription on supercoiling; (C) a typical stem-loop structure containing unpaired and mispaired bases; (D) mutation 1, a C-to-T transition in the loop.
percoiling-induced conformational changes may be required for structural changes in regulatory complexes and for recognition by RNA polymerase (RNAP) (85). Transcription has a profound effect on supercoiling, because RNAP distorts and destabilizes dsDNA. As indicated in the twin-domain model (Fig. 1B) of Liu and Wang (61), negative supercoiling is generated behind, and positive supercoiling in front of, the advancing RNAP transcription complex. Many investigations provide evidence demonstrating that transcription drives supercoiling in vivo (1, 19, 20, 27, 86) and that the wave generated can be as long as 800 bp (47). Negative supercoiling induces and stabilizes a transition from the right-handed B-form to the left-handed Z-form of DNA (42); a chemical assay detecting these distortions reveals that transcription-induced supercoiling is highly localized (47, 86). During the induction of transcription, supercoils are found inside each transcribed region, as well as upstream and downstream of each individual RNAP complex. Transcription from a strong promoter leads to greater negative supercoiling than transcription from a weak one (27). A major role of DNA topoisomerase I is now considered to be the relaxation of local negative supercoiling during transcription, thus preventing unacceptably high levels of supercoiling and associated R-loops that form when nascent RNA moves behind the advancing RNAP to bond with its original template DNA (69, 70, 105). The bulk of plasmid DNA does not exhibit stem-loop conformations during logarithmic growth. However, supercoiling may play a particularly important role in stressed cells, in which a disruption can occur between transcription and translation, thus promoting both R-loop formation and supercoiling (68, 69). The inhibition of protein synthesis by chloramphenicol, which uncouples transcription and translation, consistent with the selective derepression of relatively few genes in the absence of a single amino acid.

In response to starvation for any essential metabolite, the immediate problem is addressed specifically (e.g., derepression of a higher-affinity transport system for that metabolite), coupled with a general increase in stress resistance. Starvation results in derepression, and transcription drives localized supercoiling; the formation of stem-loop structures at regions of high superhelicity results in localized hypermutation (Fig. 1). Although energetic considerations do not favor the creation of complex structures in metabolically inactive dsDNA, transcription clearly accelerates supercoiling and transitions to secondary DNA structures (1, 19, 20, 27, 47, 59, 69, 70, 81, 86).

SECONDARY DNA STRUCTURES: ARE THEY PRECURSORS TO MUTATIONS?

Almost 40 years ago, Benzer (5) demonstrated that the mutability of specific sites in the genome varied by orders of magnitude, suggesting that these differences in mutation rate might reflect particular characteristics of the DNA sequence associated with hot spots. In fact, mutable sites are frequently the consequence of their location within DNA secondary structures. Simple stem-loops arise from ssDNA sequences containing two segments that are inverted complements, usually about 10 to 15 bases long, separated by 5 to 10 noncomplementary bases that become the loop at the end of the stem formed by hydrogen bonding of the two complementary segments (Fig. 1C). These structures are called hairpins if the loop is very small and cruciforms if they form opposite one another in each DNA strand. Perfect complementarity (a palindrome) is rare; the more common quasipalindromes or stem-loops contain bases that are left unpaired or mispaired and therefore vulnerable to deamination (mutations 1 and 2 in Fig. 1C), deletion (mutations 3 and 4), replacement (mutations 5 and 6), or complementation by the insertion of a new base to the structure (mutation 7). The stem-loop is in dynamic equilibrium with linearized DNA, and changes such as those indicated in Fig. 1C only become fixed as mutations in the course of further metabolic events such as repair or replication (Fig. 1D). For example, the entire structure depicted in Fig. 1C will be excluded and deleted by virtue of new DNA synthesis across the base of its stem. However, if this structure returns to its linear form prior to new DNA synthesis, the potential changes indicated above can be immortalized due to synthesis templated by
the altered sequence. An example of this process is indicated in Fig. 1D, in which a C in the loop is deaminated to uracil, which codes for A, which then codes for T during DNA synthesis, resulting in a C-to-T transition.

What is the probability that these structures actually exist in vivo and constitute precursors of background mutations in nature? One kind of evidence for their existence is the striking correlation of deletion end points with tandem repeats and the ends of potential secondary structures. The *leuB*, *argH*, and *pyrD* mutations in *E. coli* all occur at the end of predicted stem-loops (111). The *lacI* gene has frequently been used as a model system for investigating these correlations by comparing its nucleotide sequence (26) to those of various mutant strains. Among the sequenced deletion mutations in *lacI*, 7 occur in tandem repeats, 6 consist of deleted stem-loop structures, and 4 of these 13 mutations share both characteristics. Although three remaining mutations fail to coincide exactly with predicted vulnerable sites, they may be explained by different DNA secondary structural intermediates including interstrand misalignments (26, 88, 92). Todd and Glickman (99) analyzed 102 amber and 71 ochre mutants in the *lacI* gene, correlating mutation hot spots with the locations of predicted unpaired sites, many of which are located in stem-loop structures. Mutilational hot spots are highly localized, and 50% of the nonsense mutations arose in a segment comprising only 6% of the DNA sequence analyzed. Each hot spot is found to be located at an unpaired site within potential secondary structures. Clearly, these correlations depict causal relationships.

The mechanism of frameshift mutagenesis has also been examined in vitro during DNA polymerization (80). In this system, sequence misalignments result from intrastrand complementary pairings between two segments within a single newly synthesized strand, as well as from interstrand pairings between a segment in the new strand and a complementary sequence in the template strand. When these misaligned segments are used as templates for DNA synthesis, mutant sequences are produced. Polymerase pausing, or the local rate of DNA polymerization, was also measured and correlated with the misalignments, since pausing is sequence specific as well (43). Pausing serves to increase the time of exposure of mutagenic bases and is known to promote mutagenesis (2, 3). The correlation between pausing, positions of frameshift misalignments, and subsequent deletions can account for 97% of the mutations observed. Moreover, the most common mutations coincide precisely with the strongest pause sites, and the termini of pause sites correlate with the sequence at which the deletions begin.

Thus, in vivo and in vitro investigations strongly implicate the existence of DNA secondary structures as mutagenic substrates and/or as structural precursors to mutagenic substrates that give rise to mutations that are immortalized during new DNA synthesis or repair (Fig. 1D). As discussed earlier, mutations occur preferentially in ssDNA and in unpaired and mispaired bases (28, 31, 32, 48, 60). Other kinds of evidence also support a role for secondary structures as precursors of mutations. Many insertion mutations (Fig. 1C) can best be explained if the other strand of a predicted transient stem were used as a template for DNA synthesis prior to replication. The fact that mutations are grouped closely together much more frequently than could occur by chance implicates a single initiating event (structure). As the researchers of the above investigations point out, other variables undoubtedly contribute to and modify the correlation observed between DNA sequences, misaligned structures, polymerase pausing, and mutations. Nevertheless, these investigations are but a small fraction of an enormous literature providing compelling evidence that the sequence-dependent secondary structures created and stabilized by supercoiling are precursors to mutations.

**CONCLUSIONS**

Many scientists may share Dobzhansky's intuitive conviction that the marvelous intricacies of living organisms could not have arisen by the selection of truly random mutations. This minireview suggests that sensitive, directed feedback mechanisms initiated by different kinds of stress might facilitate and accelerate the adaptation of organisms to new environments. The specificity in the series of events summarized by Fig. 3 resides entirely in the first step, which is meant to suggest a pattern of derepression elicited by a corresponding pattern of adverse conditions. Microorganisms in nature must be confronted simultaneously by a complex set of problems, for example, the threat of oxidative or osmotic damage together with suboptimum concentrations of many essential nutrients. Transcriptional activation of genes derepressed to various degrees would expose the nontranscribed strands to mutations and stimulate localized supercoiling. Vulnerable bases in the complex DNA structures resulting from supercoiled DNA will also contribute to localized hypermutation in the genes activated to cope with the stresses that initiate the above series of events.

A multitude of random mechanisms result in hypermutation under conditions of environmental stress and clearly contribute to the variability essential to evolution. However, since most mutations are deleterious, random mechanisms that increase mutation rates also result in genomewide DNA damage. Among microorganisms, from phage to fungi, the overall mutation rate per genome is remarkably constant (within 2.5-fold), presumably reflecting an obligatory, delicate balance between the need for variation and the need to avoid general genetic damage (24, 45, 57). Thus, mutator strains are not selected in nature but remain at 1 to 2% of the population (35, 52); under certain adverse conditions, they flourish for short periods but are then selected against, apparently because of widespread deleterious effects intrinsic to genomewide hyper-
mutation. In contrast, hypermutation that is the consequence of starvation-induced derepression and transcriptional activation represents a very rapid and specific response to each adverse circumstance. The extent to which normal background mutations in nature are due to derepression mechanisms is difficult to estimate, but the location of most C-to-T transitions on the nontranscribed strand suggest that it may be significant. Regardless, a mechanism that limits an increase in mutation rates to genes that must mutate in order to overcome prevailing conditions of stress would surely be beneficial and therefore selected during evolution.

The environment gave rise to life and continues to direct evolution. Environmental changes are constantly controlling and fine-tuning the transcriptional machinery of the cell. Feed-back mechanisms represent the natural interactive link between an organism and its environment. An obvious selective advantage exists for a relationship in which particular environmental changes are metabolically linked through transcription to genetic changes that help an organism cope with new demands of the environment. In nature, nutritional stress and mental changes are metabolically linked through transcription between an organism and its environment. An obvious selective fine-tuning the transcriptional machinery of the cell. Feeding conditions of stress would surely be beneficial and therefore selected during evolution. Regardless, a mechanism that limits an increase in mutation rates to genes that must mutate in order to overcome prevailing conditions of stress would surely be beneficial and therefore selected during evolution.


